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EXPERIMENTAL INDUCTION OF INTERMALE AGGRESSIVE BEHAVIOR IN LIMBIC EPILEPTIC RATS BY WEAK, COMPLEX MAGNETIC FIELDS: IMPLICATIONS FOR GEOMAGNETIC ACTIVITY AND THE MODERN HABITAT?

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In three separate experiments, groups (4/group) of male rats with limbic epilepsy were exposed for 80 min every 24 hr during the midscotophase for 24 successive days to sham-field conditions or to one of four complex patterns of magnetic fields whose average intensities ranged between 20 nT to 500 nT. The numbers of episodes of boxing, biting, mounting, eating, drinking and grooming were then recorded each night during the latter 20 min. Moderately strong statistically significant interactions occurred between the presence or absence of the field and the pattern of the field explained 25% and 50% of the variance in the numbers of biting and boxing responses, respectively. Other behaviors were not affected. The results suggest that group aggression can be increased or decreased as a function of the temporal characteristics and morphology (shape) of the applied magnetic field.

Keywords: Magnetic field; aggression; agonistic behavior; rats; brain; shape-of-field; groups

According to Lorenz (1967), aggression is the most important behavior that allows survival of a species. The brain bases of aggression in mammals involve many structures that include the amygdala, hypothalamus and limbic cortex. If we could experimentally control the occurrence of aggression, then we might be able to understand the interactions between

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the endogenous variables, conspicuous environmental stimuli, and subtle exogenous factors that control this powerful behavior. A recent study by Michon and Persinger (1997) showed that the frequently reported correlation between increases in the weak, time-varying activity of the earth's magnetic field (geomagnetic storms) and epileptic seizures could be simulated by the experimental production of complex magnetic fields. The purpose of the present study was to discern if *specific* complex magnetic fields, whose temporal patterns of delivery during a one hour period approximate geomagnetic patterns, could be designed to specifically increase or decrease agonistic (aggressive) behavior in groups of rats with electrically labile brains.

We consider the brain as an electromagnetic matrix. Our working model is that complex electromagnetic fields applied to a conductor such as the brain should induce *temporal* patterns of small currents within but not equally throughout this matrix. The more "conductive zones" or subspaces, enhanced by loss of inhibitory input due to epileptic seizure-induced damage, should be more affected by these temporal patterns of induced microcurrents. Because the transmission of information between neurons effectively *is* the temporal pattern of electrical current, then changes in behavior associated with these microareas within the brain matrix should occur. Because: (1) specific nuclear groups within the amygdala that are known to mediate aggressive behaviors are damaged by the lithium/pilocarpine-induced seizures (Persinger, Bureau, Kostakos, Peredery and Falter, 1993), and (2) the densities of neurons within amygdaloid nuclei that are inhibited by the damaged neurons are positively correlated with the frequency of aggressive behaviors (Desjardins and Persinger, 1995), exogenous stimulations of these regions by the appropriate magnetic fields should produce or suppress aggression depending upon the pattern of stimulation.

Intuitively, weak magnetic fields within the nanoT to MicroT range, particularly if they contained the optimal temporal morphology or "time-shape" to allow interaction with the "language" of groups of neurons, should be most effective with brains that are already electrically labile. Repeated presentations of frequency-modulated patterns for 15 min have been shown to increase electrical lability as inferred by increased incidence of spontaneous seizures (Persinger, 1998). These patterns should also encourage aggression. Fields whose temporal morphologies change every few minutes should inhibit such neuronal recruitment and should decrease the amount of agonistic behavior.

METHOD

Subjects

In three separate blocks, a total of 12 male Wistar rats, approximately 90 days of age, and obtained from Charles River (breeders), were employed as subjects. The protocol for the experiment was approved by the University's Animal Care Committee.

Procedures

Each rat was first injected subcutaneously with 3 mEq/kg of lithium chloride and then injected four hours later (sub.cu.) with pilocarpine (30 mg/kg). One hour later, about thirty minutes after the rats showed the typical rearing, forepaw movements and falling (Persinger *et al.*, 1993), each rat was injected with 25 mg/kg of acepromazine to facilitate survival. During the next three days the rats were attended frequently and given food mush in water. Between one week and 10 days later the rats were then placed in the experimental, plastic cage (140 cm by 33 cm by 33 cm).

Two identical Helmholtz coils, created by wrapping 39 cm × 39 cm plastic cartons with 305 m of 30 AWG (Belden #9978) wire in a single layer (18 cm wide), were adjacent to each end of the cage. It was divided into four compartments by removal transparent plastic doors. One rat was placed in each of the four compartments. Because there were three experiments (blocks) in this study, the seizures were induced in groups of rats during three different successive months. Food and water were always available for the rats during the entire period of the experiments. The quarter-inch corncob bedding was changed about once per week. The light dark cycle was 12 : 12 with the local dark time beginning at 8:00 P.M. and the day cycle beginning at 8:00 A.M.

After a few days of habituating to the new cages, the experiment began. For each successive night for a total of 24 nights the rats were exposed to the procedure for one hour between 2300 hrs and midnight. For six successive nights the rats in each experiment were exposed to one of four types of magnetic field patterns. Each pattern was given in two successive blocks. The sequence of presentation for all of these fields were: left coil, right coil, no field, left coil, right coil, no field. This meant that there were two successive nights with fields present (but opposite side of the cage) and one night with no field present for each block. For those nights when no field

was actually presented, the computer was still activated and a dummy file was generated. The magnetic field sensor (sensitivity for 10 Hz to 100 Hz, > 10 nT) indicated no detectable field was generated.

There were five temporal characteristics to our magnetic field patterns: (1) the pulse form or signal, which was defined by a series (between 1000 and 10,000) of numbers between 0 and 255 that were translated into voltage (and magnetic field), (2) the duration of each point or pixel (usually 1 to 3 msec), (3) the time between successive presentations of the signal, the interstimulus interval (or mark-space ratio), (4) total duration of the envelope (signal and interstimulus interval) before the end of the experiment or another stimulus envelope was presented, and (5) the numbers of different program or envelopes per daily exposure.

The pulse forms that were produced during the one hour nightly exposures were called: frequency modulated (Thomas *et al.*, 1997), the fast frequency pattern (composed of 10,000 points) designed to inhibit seizures, a batch file that was composed of 19, sequentially presented (Persinger, Richards and Koren, 1997), different sine-wave and asymmetric patterns (each lasting about 2 to 3 min) and the electromagnetic signature for the word "mother" (the DOS numerical series employed to generate these patterns can be sent by e-mail upon request). The latter pattern served as a control for non-specific electromagnetic field effects. The shape and spectral profile of the fast frequency pulse is shown in Figure 1.

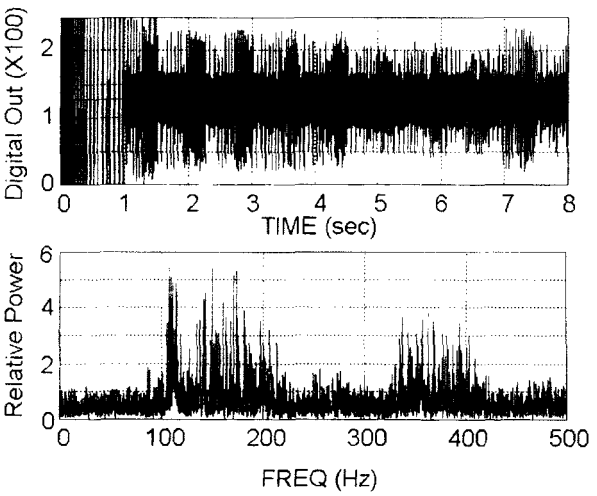

FIGURE 1 General shape (over about 30 sec) of the fast frequency pattern (10,000 pixels per presentation) and the relative power (magnitude) of the spectral analysis of this pattern (presented at 1 pixel per msec) employed in this study.

The different envelopes of the batch file contained our most effective field patterns from the last 10 years of research that included a long-term potentiation pulse (Persinger, Richards and Koren, 1994), sine-wave frequencies, *e.g.*, 1.5 Hz, 5 Hz, 10 Hz, 40 Hz, and a pattern whose expanding interpulse time (in msec) within the signal employed the Fibonacci series. The pixel duration for each point for the frequency modulated and "mother" patterns were 3 msec and the interstimulus time (time between presentation of successive signals) was 3 sec. The pixel duration for the fast frequency pattern was 1 msec; the 19 different pulse forms within the batch file included both 1 msec and 3 msec pixel durations and the interstimulus intervals were either 1 msec or 500 msec. The port latency per pixel for the Zenith XT was 190 microsec.

These four patterns were selected by the second author, without the first author's knowledge (to avoid observation bias). All patterns were generated by a Zenith XT personal computer and delivered through a custom-constructed digital to analogue converter to the appropriate coil. To be sure that simply time or duration in the cage (24 days) did not affect the rats' behaviour, each of the three experiments started with a different pattern of the four patterns. The mean and standard deviations for the intensities of the fields as measured by a sensor sensitive to 10 nT for the four exposure compartments were (closest to the activated coil to furthest): 510 and 170 nT, 93 and 25 nT, 32 and 7 nT, and, 21 and 5 nT.

After the one hour of exposure the doors of the cage were opened and the rats were allowed to interact for 20 min. To observe the rats, the overhead red fluorescent light (< 5 lux) was activated. During this period, the numbers of bites (defined as dental contact with another rat), boxing (erect stance with forepaw movements towards another rat), mounting (pelvic thrusting of another rat), eating, drinking, and grooming for each rat was recorded. Inter-rater reliability has been at least 0.90. The Cronbach alpha coefficients for reliability across (24) days exceeded 0.80 for all measures.

The data were analyzed by SPSS software. The basic analysis was a four way analysis of variance with one level not repeated (the intensities, *i.e.*, the four cage distances from the activated coil) and three repeated (the field pattern, the block, *i.e.*, first 3 days, second 3 days , and the presence or absence of the field). *Post hoc* analysis was done by combining those within-subject variables that were indicated by the results of the MANOVA. To accommodate possible contributions from daily, global geomagnetic activity, the values (in nT) for Mayaud's aa (average antipodal index) values for the 24 hr periods (Geomagnetic Indices Bulletin, National

Geophysical Data Center, 325 Broadway, Boulder, Colorado 80303) for all of the observation days were employed as covariates.

Because there may have been differences between the three groups of rats, due to age or some unidentified feature of inducing seizures within the rats, z-score transformations were done. This was completed by obtaining the mean of all of the scores for each of the 24 days of the experiment and the standard deviation for these measures. The z-score for each day and therefore each pattern was obtained by subtracting the raw score from the mean score of that measure and then dividing by the standard deviation. z-scores were obtained for all of the above behavioral measures.

RESULTS

Four way analyses of variance were completed for all of the behavioral measures as a function of: (1) the type of pulse form: (a) the frequency modulated (Thomas) pulse, (b) the high frequency pulse, (c) the batch sequence, and (d) the electromagnetic equivalent of the spoken word "mother", (2) block (the first 3 days vs the second 3 days of each pattern), (3) the presence of the field: (a) left coil, (b) right coil, and (c) no field and, (4) the rats' cage position during the first hour of exposure (intensity).

The results of the four way analysis of variance with three levels repeated (type of magnetic field pattern, block, and field present *vs* field absent) and one not repeated (intensity of the field before the social interaction) for the mean numbers of episodes of boxing and biting during the first 20 min for the 24 successive night sessions displayed a statistically significant interaction (all dfs = 6, 60) between the type of magnetic field pattern and the presence or absence of the field for the numbers of boxing events [$F = 7.04$, $p < 0.001$; eta-squared = 42%] and biting events [$F = 5.96$, $p = 0.02$; eta-equared = 26%] only. The Z-transformations for this interaction for boxing [$F = 7.04$, $p < 0.001$; eta-squared = 47%] and biting [$F = 2.96$, $p = 0.01$; eta-squared = 26%] did not appreciably change the strength of the effect. This interaction was not statistically significant for the numbers of z-transformed episodes for eating, drinking, grooming or mounting.

Post hoc analysis for both the mean numbers of boxing episodes and the numbers of bites indicated that two of the field patterns (frequency modulated, batch) being on or off were the primary source of the statistically significant interactions (see Tab. I). The nights during which the frequency modulated pulse was present was associated with significantly more boxing

TABLE I Means and standard deviations for the numbers of boxing and biting episodes as a function of the pattern of the field and the activation of the left or the right coil (field on) or no field (field off)

Boxing

Pattern	*Left coil*		*Right coil*		*No field*	
	M	*SD*	*M*	*SD*	*M*	*SD*
Frequency Mod	12.6	6.1[b]	12.0	5.5[b]	9.1	6.4[a]
High Frequency	9.3	8.3	9.8	8.5	14.6	7.5
Batch Sequence	8.0	5.4[a]	6.8	6.0[a]	12.6	7.9[b]
EM "Mother"	12.5	7.4	13.6	8.1	13.6	9.5
Biting						
Frequency Mod	0.5	0.6[b]	0.4	0.6[b]	0.1	0.1[a]
High Frequency	0.4	0.6	0.5	0.8	0.4	0.6
Batch File	1.1	0.7	0.3	0.2[a]	0.6	0.2[b]
EM "Mother"	0.7	0.8	0.6	0.6	1.0	0.9

[a] *vs.*
[b] $p < 0.05$, *post hoc*.

(for left or right coil) than those nights when there was no field present. On the other hand, the batch file pattern was associated with the opposite effect. On those nights that the batch file pattern was present the rats displayed less boxing and biting than during the nights when the field was not present. The frequency modulated pulse increased the biting while the batch file pattern decreased this agonistic behavior only when the right coil was activated.

Removal of the shared variance between ambient geomagnetic activity on the days of the observations did not significantly change the strength of the interaction (all dfs = 6, 47) between the pattern of the field and presence or absence of the field for the raw scores of boxing [$F = 6.75$, $p < 0.001$; eta-squared = 46%] or biting [$F = 2.58$, $p = 0.02$; eta-squared = 25%] or the z-transformations for boxing [$F = 8.00$, $p < 0.001$; eta-squared = 51%] and biting [$F = 2.72$, $p = 0.02$; eta-squared = 24%]. The mean and standard deviations for the aa (in nT) values during the 72 observation nights (3 experiments × 24 nights) were 13.4 and 9.8, respectively. The correlations (r) between geomagnetic activity and the different behavioral measures were biting 0.35 ($p < 0.05$), boxing 0.24, grooming −0.12, drinking 0.06, eating −0.05, and mounting −0.10.

DISCUSSION

To our knowledge this is the first report of the experimental control of intermale agonistic behavior, defined as increased incidences of boxing and

biting, by the external application of specific patterns of weak, complex magnetic fields. Because the frequency modulated pulse increased the incidence of agonistic behavior compared to nights when there were no fields presented and a batch file (which contained a tandem sequence of 19 different patterns) decreased these behaviours, we suggest that effects were specific to the temporal shapes of applied magnetic fields. The presentation of these field patterns during different times in each experiment (block) minimized the likelihood that the effects were artifacts of the duration of the experiment or the numbers of daily interactions between rats.

Comparison of the means for the episodes of boxing and biting for the no-field nights and field nights during the application of the frequency modulated pulse and the batch file, which were primarily responsible for the interaction between the patterns of the fields and their presence or absence, indicated the fields may have been modulating intrinsic variables that generated the agonistic behaviours between the males. The mean numbers of agonistic behaviours for the nights during which the frequency modulated pulse was given was not significantly different from the no-field nights after the inhibiting effects of the batch file fields were observed. This result suggests that compensatory overshoots and undershoots of behavior may occur following the brief (6 ksec) presentations of weak magnetic fields.

The fact that boxing and biting were significantly affected by the application of the fields while the incidence of grooming, eating, mounting and drinking were not, also indicates that the effects of the treatments were specific for agonistic behaviours. The strength of the effect for agonistic behavior was also indicated by its persistence over three separate experiments, two separate blocks per field per experiment and its detectability even when each rat was used as its own control (z-score transformations).

Complexity of the field, *per se*, did not modulate the agonistic behavior. The repeated application of the electromagnetic signature for the word "mother" did not produce any changes in aggression relative to the no-field condition. We could not address potential interactions between the duration of exposure and the intensity of the field exposures. In the present study rats were exposed to the fields for one hour before the observation and during the 20 min of observation. During the latter period the rats were allowed free range and hence were exposed to all of the field intensities. More recent studies have suggested that exposure for one hour before (but not during) social interaction produces much stronger effects that reflect the intensity dependence of the field which in this study was determined by the distance from the active coil. This design is more ecological relevant because the

distal two positions from the activated coil displayed field strengths that are comparable to those associated with geomagnetic activity.

That artifacts from concurrent geomagnetic activity did not confound our results was indicated by their persistence even after the daily averages for geomagnetic activity (aa values) were first removed from the analysis. Average daily geomagnetic activity, particularly values over 20 nT, have been associated with marked increases in intermale aggression (Persinger, 1997; St-Pierre and Persinger, 1998). The primary difference between the correlations with geomagnetic activity and the measurements during the application of experimental fields is that the latter's largest effect size was upon boxing behavior while the former's greatest effect is upon the biting behavior. Consequently we have not replicated the exact simulation of natural conditions for specific agonistic responses that was demonstrated for the display of nocturnal limbic seizures (Michon and Persinger, 1997).

Male rats with limbic epilepsy (induced by a single systemic injection of lithium and pilocarpine) were used as subjects because their aggressive behaviours are more frequent than normal rats. Rats with this history of neuroelectrical lability were also selected because our theoretical model predicts that the disinhibited neurons within the central nucleus of the amygdala and its functionally connected structures within these rats would be more susceptible to "driving" and "recruitment" by externally applied fields. This pattern has been observed for human beings who display temporal lobe lability (Persinger, 1998; Persinger *et al.*, 1997).

The metaphor we have pursued is that a more malleable neuromatrix, within which the capacity for reactive inhibition or countercurrents have been attenuated by excitotoxic damage, allows more direct production of fields of microcurrents within subspaces or "patches" of the cerebral volume. Whether or not the actual shape of these complex, induced fields within the brain are actual reproductions of our applied fields or modifications due to substractions and summations with the endogenous fields, *e.g.*, "beat frequency patterns" remains to be determined. Our field measurements in some houses whose owners report irritability and other "clinical symptoms" indicate that complex temporal ("beat") patterns from interactions of multiple power-frequency sources from specific geometric arrangements of electrical appliances and external sources, such as transformers and powerlines, can be created within the home environment.

We may have observed a similar phenomenon in the present study. Although which coil was activated on any given night did not affect the magnitude of the field effect for boxing behaviors, an asymmetry for the coils was noted for biting. Inspection of the experimental arrangement

indicated that the left coil (which was not associated with suppression of biting) was 1 m under the computer that generated the complex fields and may have produced "beat patterns". We also found that rats who were housed in the cage area (vertical field strength = 50 to 70 nT) immediately under the XT computer displayed significantly more grooming (an indicator of release of adrenocorticotrophic hormone) than did rats housed in the more distal two cage positions, regardless of the presence or absence of the experimental fields. Recent unpublished observations by R. O'Connor in this laboratory clearly showed that fields, within the 50 nT to 100 nT range, generated by computers, can interfere with the attenuating effects of pulsed magnetic fields upon stress-induced analgesia. These data reiterate the observations reported earlier (Persinger, 1996) that ambient magnetic fields from electronic equipment (*e.g.*, computers, transformers, oxygen pumps and operant equipment) penetrating through walls into the experimental area should be realized.

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